

Feto-Maternal Immunologic Dialogue



C.S.P.P

Part II

Humeral and Cellular Immunologic Response in EPH Gestosis

By

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ABSTRACT

This study is the part II to the part I review of the feto-maternal immunologic dialogue as a cause of EPH-Gestosis. The study is to proof the reversal of Th1, Th2 cell response in EPH-Gestosis. The IL-18 and IL12 and Th1 and Th2 ratio in 50 pregnant ladies with various degrees of EPH-Gestosis as well as 25 normal pregnant subjects in their third trimester matched to resemble demographically the diseased ladies. The results obtained confirms that IL18 in EPH-Gestosis were significantly higher than normal pregnancy. The IL12 wasnot significantly increased in mild EPH-Gestosis while it is significantly increased in severe cases. The Th1- Th2 ratio denotes significant increase and even the increase correlates with the severity of EPH-Gestosis.

Introduction:

On the assumption that the real aetiology of the syndrome of EPH gestosis is unknown yet. On searching for the real cause of this syndrome as a vascular endothelial damage that triggers the cascade of reactions evidenced by hormonal metabolic^[1,2], enzymatic^[3], immune interleukins response^[4] í gve.'hng'vj g'ecuecf g'qh' thrombus formation. This endothelial damage may be triggered by imbalanced immuno-response to fetal ingredients derived from the immunologically foreign father to the mother carried to the mother immunosystem by the deported chorionic villi^[4].

Pregnancy represents the growth of an allograft where fetal trophoblast cells evoke immune rejection and invade maternal tissue^[5,6,7]. There should be a balance between fetal trophoblast and maternal immune responsive cells. Alterations in the proportion of these cells may relate to pregnancy disorder such as pre-eclampsia. Indeed, histological changes in the placental beds of women with pre-eclampsia were noted to resemble those of rejection^[7].

The heavy traffic of the fetal DNA in the maternal blood of the EPH gestotic ladies may carry a signal of the faith to this etiologic basis of the EPH gestosis.

Saito and his colleagues^[19] calculated the percentage of Th₁, Th₂, Th₀ cell ratios of peripheral blood from normal pregnant subjects and preeclamptic patients using flow cytometry. Peripheral blood mononuclear (PBMC) cells from these subjects and patients were cultured with phytohaemagglutinin stimulation. The IL4

& IFN- γ concentrations were determined in the supernatant by ELISA, they found that the percentage of Th₁ & Th₂ and the ratio Th₁:Th₂ as well the cytokine secretion level (IFN- γ and IL-4) were increased in the 3rd trimester and also Th₁ dominate Th₂ in preeclamptic patients contrary to that in normal pregnant subjects.

Aim of the Work:

To confirm the above findings and to get an evidence for the disturbed immunologic response as evidenced by reversed dominance of Th₁:Th₂ in normal pregnant subjects and EPH gestotic pregnant patients we performed this piece of work.

Materials and Methods:

Subjects:

Five ml. peripheral blood samples were collected from:

- (1) 25 normal pregnant women with mean gestational age 34.1 weeks, mean S.D \pm 1.3.
- (2) Fifty pregnant patients with EPH gestosis were classified according to the El-Kabarity modified gestosis index^[2] as follows:
 - (a) 25 patients with mild EPH gestosis and
 - (b) 25 patients with severe EPH gestosis.

EPH gestotic patients were considered as mild, with persistent systolic blood pressure of 140 mmHg or more and/or diastolic blood pressure of 90 mmHg or more and/or odema grade 1,2 and/or prateinuria of more than 300 mgm/litre of urine, as severe EPH gestosis with persistent systolic, blood pressure of 160 mmHg or more and/or diastolic blood pressure of 100 or more, odema 1,2 & 3 after 6 hours of rest and proteinuria 1,2,3^[2].

The study was approved by the ethical committee of Ain Shams Maternity hospital and informed written consent was obtained from all patients and subjects before sampling.

Methods:

1. IL-18 and IL-12 secretion by non stimulated peripheral blood mononuclear cells (PBMC) was estimated by ELISA as a marker of humeral mediated immunity.

Peripheral blood mononuclear cells were isolated by Ficoll-Hypague gradient centrifugation. PBMC were suspended at 1×10^6 cells/ml in PRMI medium supplemented with 10% fetal calf serum and were incubated for 24 h at 37 °C in a humidified atmosphere with 5% CO₂. Supernatants were determined by enzyme linked radioassay. The limit of sensitivity for detection of IL-18 was 0.1 pg/mL. All samples were run in duplicate.

2. Flow cytometric analysis staining of intracellular and surface antigens. The percentages of Th₁ cells and Th₂, the Th₁/Th₂ ratios were determined by flow cytometry^[7,10].

Statistical Analysis:

Values are presented as means \pm standard deviation S.D. Differences between EPH gestotic patients & healthy pregnant subjects were analysed by the Mann-Whitney U-test and Fisher's Z transformation test. A value of P<0.05 was considered to indicate statistical significance.

Demographic Characteristics of Subjects

	Normal pregnancy (n=25)	Mild PET (n=25)	Severe PET (n=25)
No of primigravida	18	13	18
age in years	23 \pm 3.1	27 \pm 3.8	26.7 \pm 3.4
Gestational age at blood sampling (weeks)	34.1 \pm 1.3	34.1 \pm 3.8	32.3 \pm 3.4
Blood pressure			
Systolic mmHg	111 \pm 7	146 \pm 9**	179 \pm 13**
Diastolic mmHg	63 \pm 7	95 \pm 7**	118 \pm 9**
Proteinuria (0 1 2 3)*	0	1.8 \pm 3**	2.6 \pm 4**
Odema (0 1 2 3)	0	1.2 \pm 3**	2.9 \pm 4**

* According modified gestosis index

** Values are presented as means + standard deviation $P < 0.001$ compared with the normal pregnancy values.

Results:

IL-18 concentration in culture supernatants of PBMC from healthy pregnant women, mild and severe EPH gestotic patients were 162.3 ± 64.4 pg/mL (range of 40 - 430); 201.6 ± 56.4 pg/mL (range 50 - 400) and 212.1 ± 69.6 pg/mL (range 50 - 280), respectively.

IL-18 secretion in mild & severe EPH gestotic patients were significantly higher than its secretion in normal pregnant subjects in the 3rd trimester (P.value = 0.026 & 0.011 respectively).

IL-12 concentrations in culture supernatants of PBMC from healthy pregnant women, and mild & severe EPH gestotic patients were 0.14 ± 0.03 pg.mL (range 0 - 0.80); 0.17 ± 0.08 pg/mL (range 0 - 0.80) and 2.04 ± 0.73 pg/ml (range 0.10 - 10.50), respectively.

IL-12 secretion by PBMC was not significantly different between normal pregnant subjects and mild EPH gestotic patients while the IL-12 secretion is significantly higher in severe EPH gestotic patients than those of normal pregnant subjects (P=0.001);

Th₁/Th₂ ratios in mild EPH gestosis is significantly higher than in healthy pregnant women (P=0.02), and were further elevated in severe EPH gestotic patients.

Discussion:

Adaptive immune systems can be classified as cell mediated immunity and humoral immunity. CD4 T-cells are classified as Th₁ cells, which sensitize interleukins (IL-2), interferon IFN- γ and tumor necrosis factor TNF- α , and induce cellular immunity, or Th₂ cells, which synthesize IL-4, IL-5, IL-6, IL-10 and IL-13 and induce antibody production^[10].

Dominance of Th₁ or Th₂ can be elevated in terms of ratios of cytokines produced by T-cells^[11].

The present study has examined IL-18 and IL-12 by non stimulated PBMC in normal pregnant women in their 3rd trimester and EPH gestotic patients , both mild & severe types.

Although IL-18 mRNA is expressed in a wide range of cells including monocytes/macrophages, kupffer cells, T-cells, B-cells, dendritic cells, osteoblasts, keratinocytes and astrocytes, the main producers are monocytes/macrophages^[12,13 & 14].

Since the numbers of PBMC increased in normal pregnant subjects and preeclamptic patients^[15], increased number of PNMC might contribute to the elevated IL-18 secretion.

Several recent reports indicate that IL-18 is expressed at sites of chronic inflammation^[16,17].

Our study demonstrated that Th₁ - type cytokine production predominates in patients with EPH gestosis. In our study, we have measure IL-12 secretion by non stimulated PBMC, because we wished to determine the cytokine profile in vivo, and stimulation by endotoxins did not occur in EPH gestosis.

It is suggested that IL-12 secretion by non stimulated PBMC is suppressed in normal pregnancy, and the suppressed IL-12 secretion might induce Th₂ dominant immunological environment in normal pregnancy but when microbes invade the maternal body or antigens such as fetal cell, into maternal circulation, primed monocytes may quickly produce IL-12. Indeed, a type 1 cytokines response been reported in preterm delivery which is associated with chorio-amnionitis^[18]. IL-18 and IL-12 synergistically exerts their IFN- γ inducing activities in T-cells and NK cells, where as each cytokine is much less active.

Our results suggest that changes in IL-12 against a contrast background of IL-18, in pregnancy may contribute to both the Th₁/Th₂ balance in normal pregnancy and in EPH gestosis.

Further analyses are needed to elucidate the mechanisms by which the odema, proteinuria and hypertension that occur in EPH gestosis are induced by predominance of Th₁ cytokines. Similar statement was written by *Saito et al.*^[19]

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